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Ecotoxic effects of loratadine and its metabolic and light-induced derivatives

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Abstract

Loratadine and desloratadine are second-generation antihistaminic drugs. Because of human administration, they are continuously released *via excreta* into wastewater treatment plants and occur in surface waters as residues and transformation products (TPs). Loratadine and desloratadine residues have been found at very low concentrations (ng/L) in the aquatic environment but their toxic effects are still not well known. Both drugs are light-sensitive even under environmentally simulated conditions and some of the photoproducts have been isolated and characterized. The aim of the present study was to investigate the acute and chronic ecotoxicity of loratadine, desloratadine and their light-induced transformation products in organisms of the aquatic trophic chain. Bioassays were performed in the alga *Pseudokirchneriella subcapitata*, the rotifer *Brachionus calyciflorus* and in two crustaceans, *Thamnocephalus platyurus* and *Ceriodaphnia dubia*. Loratadine exerted its acute and chronic toxicity especially on *Ceriodaphnia dubia* (LC50: 600 µg/L, EC50: 28.14 µg/L) while desloratadine showed similar acute toxicity among the organisms tested and it was more chronically effective compound in *Ceriodaphnia dubia* and *Pseudokirchneriella subcapitata*. Generally, transformation products were less active both in acute and chronic assays.

Keywords: antihistaminic drug; loratadine; desloratadine; acute toxicity; chronic toxicity; photoproducts.

Highlights

- Loratadine was irradiated by UVB and sunlight and its photoproducts were isolated and characterized.
- Loratadine, desloratadine and light-induced TPs were tested in aquatic organisms.
- Generally, transformation products were less active both in acute and chronic assays.
- Desloratadine was the most chronically effective compound in *C. dubia* and *P. subcapitata*.

1. Introduction

Drugs are continuously released as mixtures of parent compounds and metabolites and enter the aquatic environment through hospital and municipal wastewaters. Here, these mixtures may undergo transformations due to redox or light-induced reactions, hydrolysis, and other reactions leading to transformation products, in some cases more harmful than parent compounds (DellaGreca et al., 2014; Passananti et al., 2015; Isidori et al., 2016). The importance of these events in the breakdown of drugs has stimulated a large number of researches concerning kinetics, degradation mechanism, isolation and toxicity of the transformation products (Lambropoulou and Nollet, 2014). Generally, the most commonly occurring drugs in the aquatic systems are the most administered. However, some classes of drugs highly utilized by patients are not detected in the waters because rapidly degraded, while in some cases drugs less utilized are detected at high concentrations because resistant to biodegradation. Among the most administered drugs, antihistamines are detected in surface waters because of their poor removal by conventional wastewater treatments (Kosonen and Kronberg, 2009; Radjenovic' et al., 2009; Valcarcel et al., 2011) and due to their low polarity and scarce volatility, they may represent a hazard for the aquatic ecosystem (Berninger and Brooks, 2010; Kristofco and Brooks, 2017).

Among antihistamines, ranitidine, difenidramine, cimetidine and loratadine are the most detected in the effluents of sewage treatment plants and the detection of loratadine in surface waters has exceeded therapeutic hazard values (THVs) showing the need of understanding the aquatic toxicology, hazards and risks associated with this drug (Kristofco and Brooks, 2017). Loratadine is a second-generation antihistaminic drug so called because it causes less sedation and drowsiness than the first-generation antihistamines used to treat allergic reactions, approved by US Food and Drug Administration in 1993. Loratadine is a selective inverse agonist of peripheral H₁-receptors (Witiak, 1970, Peyrovi and Hadjmohammadi, 2015). It is mainly metabolized through the hepatic system to desloratadine, which is a pharmacologically active compound, deriving from the loss of carbamate moiety (Yumibe et al., 1996). **Forty percent and 42% of the ingested loratadine dose is excreted unchanged in urine and the feces, respectively** (Ramanathan et al., 2007). It has been detected in surface waters in Europe (in some Spanish river samples) in the low concentration range of 3.96-17.1 ng/L (Lopez-Serna et al., 2012) but also in wastewater effluents in Europe, North-America and Asia-Pacific with a maximum concentration of 58.5 ng/L (Kristofco and Brooks, 2017). Desloratadine has been detected in Europe with a maximum concentration of 81 ng/L (Kristofco and Brooks, 2017). Both drugs have also been recovered in lower amounts in marine water of Mediterranean coasts (Moreno-Gonzalez et al., 2015). Based on the antihistamines consumption data, loratadine and desloratadine should occur in wastewater at higher concentrations.

105 **However, loratadine has low affinity for suspended matter (octanol/water partition coefficient**
106 **log P equal to 5 for loratadine and 3.2 for desloratadine; El-Awady et al., 2013) and therefore**
107 **does not accumulate appreciably in sediments and remains in the water column (Moreno-**
108 **Gonzales et al., 2015).** Loratadine is known as photolabile, in fact it is stated that the drug should
109 be stored protected from light (Parfitt, 1999). Its UV spectrum shows an absorption band at λ 280
110 nm with a tail up to 300 nm, hence the drug is able to adsorb sunlight at ground level and to
111 undergo light-induced transformations in the aquatic compartment. While its photostability has been
112 investigated, no data on photoproducts identification are reported (Abounassif et al., 2005). In this
113 context, we have examined the photochemical behavior of loratadine and desloratadine in aqueous
114 medium under UVB and sunlight irradiation in order to isolate and fully characterized the
115 photoproducts. For this purpose, concentrated solutions, far from environmental concentrations,
116 were used (DellaGreca et al., 2014). The ecotoxicological effects of the parent drug, its metabolite
117 and its transformation products were evaluated in producers and primary consumers.

118

119 **2. Materials and methods**

120 *2.1 Chemicals*

121 Loratadine (99.4%, CAS Number: 79794-75-5) and desloratadine (99.6%, CAS Number: 100643-
122 71-8) were purchased by Kemprotec. All chemicals were used without further purification unless
123 otherwise indicated. Solvents (acetonitrile, methanol and diethylether) were of HPLC grade and
124 were purchased from Sigma Aldrich. Water was of Milli-Q quality and was obtained from a Milli-Q
125 gradient system (Millipore).

126 *2.2 Apparatus*

127 *2.2.1 Spectroscopic techniques*

128 Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Inova-500 instrument
129 operating at 499.6 and 125.6 MHz for ^1H and ^{13}C , respectively, and referenced with CDCl_3 . The
130 carbon multiplicity was evidenced by DEPT experiments. The proton couplings were evidenced by
131 ^1H - ^1H COSY experiments. The heteronuclear chemical shift correlations were determined by
132 HMQC and HMBC pulse sequences.

133 IR spectra were recorded on a Jasco FT/IR-430 instrument equipped with single reflection ATR
134 using CHCl_3 as solvent.

135 UV-Vis spectra were recorded with a Varian Cary 300 UV-Vis spectrophotometer or on a
136 PerkinElmer Lambda 7 spectrophotometer.

137 *2.2.2 Chromatographic analysis*

HPLC experiments were carried out on an Agilent 1100 HPLC system, equipped with an UV detector set at 254 nm, using a RP-18 column (Gemini, 5 μ m, 110 Å, 250 mm \times 4.6 mm). at a flow rate of 0.8 mL min⁻¹.

The analysis of the solutions used for determining the kinetic constant of loratadine photodegradation was carried out using the gradient elution as follows: at initial time 30 % acetonitrile and 70 % water for 7 min, followed by an increase of acetonitrile up to 70% in two minutes. Then, the same ratio was maintained constant for 24 minutes; finally, the initial ratio (30 % acetonitrile and 70 % water) was reached in two minutes.

In other cases HPLC analysis was performed under isocratic conditions and H₂O /CH₃CN 4:6 v/v was used as eluent.

GC-MS analyses were performed on a 6890 MSD quadrupole mass spectrometer (Agilent technologies) equipped with a gas chromatograph by using a Zebron ZB-5HT Inferno (5%-Phenyl-95%-Dimethylpolysiloxane) fused silica capillary column (Column 30 m \times 0.32 mm \times 0.10 μ m) from Phenomenex. The injection temperature was 250°C, the oven temperature was held at 50°C for 3 min and then increased to 150°C at 12°C/min, increasing to 230°C at 18°C/min, to 280°C at 10°C/min and finally to 300°C at 30°C/min and held for 3 min. Electron Ionization mass spectra were recorded by continuous quadrupole scanning at 70eV ionization energy, in the mass range of m/z 30-600.

2.2.3 Irradiation apparatus

The photoreactor (Multirays, Helios Italquartz) was equipped with six 15W lamps with a maximum at 310 nm (UV-B). Open quartz tubes (1 cm optical path) and open and closed pyrex tubes (20 cm \times 1 cm, 25 mL) were used.

2.3 Chromatographic separation materials

Analytical and preparative Thin Layer Chromatography (TLC) was made on Kieselgel 60 F₂₅₄ plates with 0.2 mm, 0.5 or 1 mm layer thickness, respectively (Merck).

2.4 Experiments

2.4.1 Stability in aqueous solution in the dark

Loratadine (**1**) solutions (1 \times 10⁻⁴ M) in H₂O/CH₃CN (9:1, v/v) at pH 4, 7 and 9 were prepared. The acid and alkaline solutions were made using NaOH 2M and HCl 2M to adjust pH level. All solutions were kept in the dark and analyzed by HPLC (isocratic conditions) at 12 h and 48 h.

2.4.2 Kinetic constant and quantum yield determination

Kinetics data were obtained by irradiating the drug (1 \times 10⁻⁴ M solution in H₂O/CH₃CN 9:1, v/v) in open quartz tubes and monitoring the solution at fixed time intervals by HPLC using the proposed gradient elution. The time evolution was fitted with a pseudo-first order equation $C_0 = C_t \times e^{-Kt}$

172 where C_0 is the initial drug concentration, C_t the concentration at time t and k the pseudo-first order
173 degradation rate constant.

174 The incident photon flux (4.98×10^{21} photons $\text{m}^{-2} \text{s}^{-1}$) in solution, used to calculate the quantum
175 yield of loratadine, was calculated using *p*-nitroanisole/pyridine actinometer (Dulin and Mill, 1982).

176 **2.4.3 Irradiation experiments.** Two solutions of loratadine in $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ (9:1, v/v, 1×10^{-4} M) and
177 $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ (75:25, v/v, 1×10^{-4} M) were irradiated in open quartz tube and analysed by HPLC at
178 selected times. The photoproducts were identified by HPLC comparing their R_t values with those of
179 standard compounds which were isolated and characterized by performing preparative
180 photochemical experiments (see below). An aliquot of the $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ (75:25, v/v, 1×10^{-4} M) was
181 analyzed by GC-MS after 6 min irradiation.

182 **2.4.4 Preparative experiments for photoproducts isolation**

183 The photoproducts were isolated by means of preparative TLCs of irradiation mixtures obtained by
184 appropriate experiments. Their structures were determined by spectroscopic analyses (Hesse et al.,
185 2008). The presence of functional groups was deduced by IR spectra and identification of all
186 different protons and carbons was obtained by NMR spectra.

187 **2.4.4.1. Isolation of isoloratadine 2**

188 Loratadine (35 mg) was dissolved in 92 mL of $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ (75:25 v/v, 1×10^{-3} M) and divided in
189 four closed quartz tubes. Each solution was saturated with argon and irradiated by UV-B lamps.
190 After 20 min of irradiation the solvents were evaporated under vacuum and the residue was
191 analysed by ^1H NMR and separated by preparative TLC. Elution with Et_2O gave a fraction
192 consisting of **3** and **4** (2 mg), isoloratadine **2** (6 mg), loratadine **1** (3 mg) and an intractable polar
193 material (11 mg).

194 *Ethyl 4-(8-chloro-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-yl)-5,6-*
195 *dihydropyridine-1(2H)-carboxylate (2):* EI-MS m/z 382/384 ; $\text{UV}\lambda_{\text{max}}$ (CH_3OH) nm 266 (log ϵ 3.8);
196 $\text{IR}\nu_{\text{max}}$ (CHCl_3) 1690 (-N-CO-O-), 1606 (stretching vibrations of aromatic rings), 1371 (C-O
197 stretching) cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 8.39 (1H, d, $J = 4.0$ Hz, H-2), 7.42 (1H, d, $J = 7.3$
198 Hz, H-4), 7.20-7.12 (4H, m, H-3, H-7, H-9 and H-10), 4.84-4.80 (2H, m, H-11 e H-3'), 4.10 (2H, q,
199 $J = 7.0$ Hz, CH_2O), 3.88 (2H, m, H-2'), 3.50-3.45 (4H, m), 2.88-2.74 (2H, m), 1.94-1.71 (2H, m),
200 1.23 (3H, t, $J = 7.0$ Hz, CH_3). ^{13}C NMR (126 MHz, CDCl_3) δ 157.0 (C-1a), 155.5 (CO), 146.8 (C-
201 2), 141.8 (C-6a), 138.5 (C-4), 135.9 (C-10a), 135.0 (C-4a), 133.1 (C-7), 133.0 (C-8), 131.1 (C-4'),
202 129.8 (C-10), 126.3 (C-9), 122.4 (C-3), 121.1 (C-3'), 62.3 (C-11), 61.2 (CH_2O), 43.4 (C-2'), 40.5
203 (C-6'), 31.3 (x2, C-5 e C-6), 28.0 (C-5'), 14.9 (CH_3).

204 **2.4.4.2 Isolation of compounds 3 and 4**

205 Loratadine (50 mg) was dissolved in 130 mL of H₂O/CH₃CN (75:25, v/v, 1 x 10⁻³ M) and irradiated
206 by UV-B lamps. The irradiation mixture was analyzed at different time by HPLC. After 40 min of
207 irradiation the solvents were evaporated under vacuum, and the residue was analysed by ¹H NMR
208 and separated by preparative TLC. Elution with Et₂O gave a fraction consisting of **3** and **4** in ca. 3:1
209 molar ratio (11 mg), tricycle **3** (2 mg), isolaratadine **2** (2 mg), loratadine **1** (5 mg) and an intractable
210 polar residue (8 mg).

211 *8-Chloro-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-b]pyridine (3)*: EI-MS *m/z* 229/231; UVλ_{max}
212 (CH₃OH) nm: 279 (log ε 3.1); IR ν_{max} (CHCl₃) 1580 (stretching vibrations of aromatic ring), 1070
213 (aryl C-halogen stretching) cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 8.35 (1H, d, *J* = 4.9 Hz, H-2), 7.40
214 (1H, dd, *J* = 7.5, 1.4 Hz, H-4), 7.20 (1H, d, *J* = 8.1 Hz, H-10), 7.16 (3H, m, H-4, H-7 and H-9),
215 4.35 (2H, s, H-11), 3.33 (4H, brs, H-5 and H-6); ¹³C NMR (50 MHz, CDCl₃) δ 156.9 (C-1a), 146.5
216 (C-2), 140.8 (C-6a), 137.7 (C-4), 136.0 (C-10a), 133.8 (C-4a), 132.3 (C-8), 130.7 (C-10), 128.9 (C-
217 7), 126.3 (C-9), 122.0 (C-3), 35.7 (C-11), 31.3 (C-5 and C-6).

218 Spectral data of piperidinone **4** were deduced by those of the mixture of **3** and **4** after the signals of
219 tricycle **3** were subtracted; it was identified by comparison of its signals with those reported in
220 literature (Hirsch and Havinga, 1976).

221 *Ethyl 4-oxopiperidine-1-carboxylate (4)* (in mixture with **3** in ca. 1:3 molar ratio): EI-MS *m/z* 171;
222 ¹H NMR (500 MHz, CDCl₃) δ 4.19 (2H, q, *J* = 7.1 Hz, CH₂O), 3.76 (4H, t, *J* = 6.1 Hz, H-2 and H-
223 6), 2.45 (4H, t, *J* = 6.1 Hz, H-3 e H-5), 1.29 (3H, t, *J* = 7.1 Hz, CH₃). ¹³C NMR (125 MHz, CDCl₃)
224 δ 207.1 (C-4), 155.0 (CO), 61.8 (CH₂O), 43.0 (C-2 and C-6), 41.1, (C-3 and C-5), 14.6 (CH₃).

225 2.4.4.3. UV-B irradiation experiments for mechanistic purposes

226 Two 1 x 10⁻³ M solutions of loratadine in pure CH₃CN were prepared by dissolving 5 mg in 13 mL.
227 A solution was irradiated in open quartz tubes and the other one in closed quartz tubes after
228 saturating with argon. After 15 min the solvent was evaporated and each residue analysed by ¹H
229 NMR.

230 A similar procedure was used for two 1 x 10⁻³ M solutions of loratadine in methanol and for two
231 solutions of loratadine in H₂O/CH₃CN (7:3 v/v).

232 2.4.4.4 Irradiation of isolaratadine 2

233 A 1 x 10⁻⁴ M solution of compound **2** in H₂O/CH₃CN (9:1 v/v) was irradiated in open quartz tubes
234 with UV-B lamps and analysed by HPLC and ¹H-NMR.

235 2.4.4.5 Irradiation of desloratadine 5

236 1 x 10⁻³ M solution of desloratadine **5** was prepared by dissolving 5 mg in 16 mL of H₂O/CH₃CN
237 7:3 v/v. The solution was irradiated by UV-B lamps and analysed by ¹H-NMR.

238 2.5 Toxicity testing

239 Samples were dissolved in dimethylsulphoxide (DMSO, 3% v/v), stored in the dark at 4 °C, further
240 diluted in deionized water (Elix 10, Millipore, Milan, Italy) and sonicated for 30 minutes to obtain
241 stock solutions. The test solutions were prepared by mixing the appropriate volumes of the stock
242 solutions and ISO test media. Toxicity assays were performed in the following organisms: the green
243 alga *Pseudokirchneriella subcapitata*, the planktonic rotifer *Brachionus calyciflorus* abundant in
244 freshwaters, the anostracan crustacean *Thamnocephalus platyurus*, highly sensitive in acute toxicity
245 testing and the cladoceran crustacean *Ceriodaphnia dubia*, worldwide distributed and often
246 employed in acute and chronic toxicity testing.

247 2.5.1 Determination of drugs concentration in test samples

248 The concentrations of drugs were **measured (n=1)** using the solid phase extraction (SPE) coupled
249 with HPLC. Each test solution containing drugs at the beginning of each toxicity test and after 24 h,
250 48 h and 72h passed through a C18 Sep-Pak® light column (Waters) used as a solid phase extraction
251 cartridge, previously conditioned with 5.0 mL methanol followed by 5.0 mL water. The cartridge was
252 then eluted with 5 mL methanol. The eluate was evaporated to dryness under reduced pressure and
253 the residue was suspended in 1.0 mL acetonitrile. Portions of 200 µL volume were then injected into
254 the HPLC system.

255 2.5.2 Acute toxicity tests

256 *B. calyciflorus* organisms were hatched from cysts (MicroBioTest Inc., Nazareth, Belgium) in
257 synthetic moderately hard freshwater (80-100mg/L CaCO₃, pH7.5 ± 0.3) at 25 ± 1 °C under
258 continuous illumination (3000-4000 lux) for 16-18 h prior to test initiation, as reported in the
259 ASTM E 1440-91 guidelines. Six replicates with five animals/well, less than 2h old, were
260 performed for each concentrations (0.3mL of test solution for each test well in 36-well plates,
261 MicroBioTest Inc., Nazareth, Belgium) of each compound.

262 The *T. platyurus* test was performed in according to ISO 14380 (2011) using larvae hatched from
263 cysts (Thamnotoxkit F, MicroBioTest Inc., Nazareth, Belgium) in 20-22 h before the assay in the
264 standard freshwater (dilution 1:8 with deionized water) at 25°C under continuous illumination
265 (3000-4000 lux). Tests were performed in 24-well plates with 10 crustaceans/well (1.0 mL of test
266 solution), in three replicates.

267 The *C. dubia* test was performed over 24 h of exposure using young organisms less than 24 h old
268 following test conditions reported in EPA-600-4-90 (US EPA 1993) with slight modifications.
269 Neonates of at least third generation coming from a healthy mass culture (starting organisms were
270 purchased from Aquatic Research Organisms, Inc., Hampton, NH, USA) were maintained at 25 ± 1
271 °C in synthetic medium (hardness 250 mg/L expressed as CaCO₃) with a 16:8 h light:dark cycle

(600 lux) Tests were performed in 24-well plates with 10 crustaceans per well (1.0 mL of test solution), in three replicates.

For each test considered above, both a negative control (only test-medium) and a solvent control (DMSO 1% v/v related to the maximum concentration of compounds tested equal to 100 mg/L) were performed. The plates were incubated in darkness at 25 °C for 24 h.

The end-point considered was mortality, and the concentration resulting in a 50% effect in 24 h-exposure was indicated as Median Lethal Concentration (LC50).

In acute assays, compounds were tested for a maximum of eight dilutions depending on the respective sensitivity of the organisms (100-31.25-9.76-3.15-0.98-0.31-0.09 mg/L) starting from the highest concentration of 100 mg/L with a geometric progression of 3.2.

2.5.3 Chronic toxicity tests

The *B. calyciflorus* chronic test was based on the offspring reduction over 48 h exposure (ISO, 20666, 2008) and was performed on young organisms less than 2 h old. Cysts were hatched as previously described for the acute test. Tests were performed in 48-well plates with one rotifer/well (0.9 mL of test solution prepared in moderately hard dilution water, ASTM E1440-91), in six replicates. The organisms were fed with a fresh suspension (0.1 mL) of 10^7 cells/mL of the unicellular alga *P. subcapitata*. Plates were incubated in darkness at 25 °C.

The chronic test in *C. dubia* was performed with female neonates < 24 h old from at least the third generation of a stock culture maintained in synthetic water with ISO medium were individually exposed to 25 mL of test solution in beakers over 7 days (ISO, 20665, 2008). Tests were conducted in semi-static conditions (all test media were exchanged five times per week) and, from the fourth day-exposure onward, the offspring produced by each parent organism were counted and removed daily. The organisms were fed daily with 200 µL of a combination of yeast *Saccharomyces cerevisiae*, alfalfa and flake food in addition to the unicellular green alga *P. subcapitata* (10^8 cells/mL). Ten replicates per concentration were incubated at 25 ± 1 °C with a 16:8 h light:dark cycle (600 lux).

The *P. subcapitata* growth inhibition test was performed according to OECD 201, 2011 with slight modifications reported by Paixao et al., 2008. The single samples were incubated with 10^4 cells/mL of algal suspension in 96-well microplates in six replicates under continuous illumination at 25 ± 1 °C on a microplate shaker (450 rpm). The plates were read at 450 nm (SpectraFluor, Tecan, Switzerland) immediately before the test and after 24 h, 48 h and 72 h.

For all chronic tests, a negative control (test-medium control) was used to the test series. Only for *P. subcapitata* growth inhibition test, the % DMSO exceeded the maximum % recommended in toxicity testing (0.01%). Thus, for this kind of assay, a solvent control (DMSO 0.1% v/v related to

the maximum concentration of compounds tested equal to 10000 µg/L) was performed. The number of the offspring or the algal growth outputs were compared to the values obtained for the negative control in order to determine the chronic effective percentages and to evaluate the chronic Effective Concentrations (ECx).

In *B. calyciflorus* and *C. dubia* chronic assays, compounds were tested for a maximum of nine dilutions (1000-312.5-97.66-30.52-9.54-2.98-0.93-0.29-0.09 µg/L) starting from the highest concentrations of 1000 µg/L with a geometric progression of 3.2. For the *P. subcapitata* growth inhibition test, compounds were tested for a maximum of ten dilutions (10000-3125-976.56-305.17-95.37-29.80-9.31-2.91-0.91-0.28 µg/L) starting from the highest concentration of 10000 µg/L with a geometric progression of 3.2.

2.5.4 Ecotoxicological data analysis

For each kind of assay, three independent experiments were performed. For each independent experiment, the effect percentages were calculated comparing each specific negative control. For each assay, the effect percentages coming from three independent experiments were pooled using Prism5 software (Graphpad Inc., CA, USA) to estimate the concentrations giving x% effect (L(E)Cx) by non-linear regression (log agonist vs. normalized response-variable slope). The LC50 value, corresponding to the 50% of mortality for each test-organism, was the test parameter for acute tests, whereas EC50, EC20, and EC10 were the concentrations giving 50%, 20% or 10% of the effect used in chronic tests to evaluate the inhibition of reproduction or the inhibition of the algal growth.

3. Results

The SPE coupled with HPLC analysis revealed a non-appreciable difference between nominal and actual concentrations: the actual concentrations of tested chemicals **diverged from the nominal concentrations by 5% after 24h, around 10% after 48h, and around 15% after 72h.** According to Li (2012), when the actual concentrations are at least 80% of the nominal concentrations, the measured and the expected concentrations are considered to be very close and no significantly different, so that in the present study the effective concentrations are reported as nominal concentrations.

3.1 Photochemical behaviour of loratadine

Loratadine **1** is slightly soluble in water, hence acetonitrile was chosen as co-solvent to obtain clear solutions (Figure 1). Preliminary experiments were carried out in the dark using 1×10^{-4} M solutions in H₂O/CH₃CN 9:1 v/v. The drug was stable after 48 h even when tested in acidic (pH 4)

and alkaline (pH 9) solutions. These pH ranges are usually considered in environmental analysis (Valenti et al., 2009).

Loratadine solution was then irradiated in a photoreactor with UV-B lamps. HPLC analysis showed the formation of photoproducts already after 2 min (Figure 2): compound **2** at R_t 16.2 min and compound **3** at R_t 10.2 min (Figure 1). The photoproducts were identified comparing their R_t values with those of standard compounds which were isolated and characterized by performing preparative photochemical experiments.

Kinetic experiment under these conditions showed that loratadine has a half-life of 137.4 seconds and a polychromatic quantum yield of 5.89×10^{-4} (Table S1).

Preparative experiments to isolate and characterize the photoproducts were carried out by UV-B irradiation of 1×10^{-3} M solutions of the drug in H_2O/CH_3CN 75:25 v/v. HPLC analysis confirmed the trend observed in dilute conditions and revealed the presence of the peaks of products **2** and **3** together with other minor products. After 40 min of irradiation, TLC on silica gel afforded three photoproducts: compounds **2** and **3** and a new product **4**. Structures **2-4** (Figure 1) were assigned on the basis of spectral data. In particular, 1D and 2D NMR spectroscopy was used because it is a powerful technique for identification and structure elucidation of small organic molecules (Elyashberg, 2015; Fuloria and Fuloria, 2013).

The mass spectrum of photoproduct **2** (R_t 16.5 min) shows a molecular peak at 382/384 m/z corresponding to the molecular formula $C_{22}H_{23}ClN_2O_2$, hence suggesting that it is a loratadine isomer. The mass spectrum evidences a peak at m/z 154, absent in the mass spectrum of loratadine, attributable to the tetrapyridine fragment $C_8H_{12}NO_2$. The 1H -NMR spectrum shows significant differences only in the aliphatic proton region. In particular, two overlapping signals due to protons H-11 and H-3' (singlet + multiplet, respectively) are observed at δ 4.80 as expected due to the shift of the double bond. The shift of the double bond produces, in the ^{13}C -NMR spectrum, the disappearance of the singlet carbon signal at δ 133.3 (C-11 of **1**) and the appearance of a doublet carbon signal at δ 120.8 (C-3' of **2**).

The structure of photoproduct **3** (R_t 10.2 min) was confirmed by the presence in the mass spectrum of molecular peak at 229/231 m/z corresponding to the molecular formula $C_{14}H_{12}ClN_2$. The 1H -NMR spectrum shows the presence of six aromatic protons in the δ range 8.40-7.10, of signals at δ 4.35 and at δ 3.33 due to di-benzylic methylene proton H-11 and to benzylic methylene protons H-5 and H-6, respectively.

Piperidinone **4** was obtained by TLC in mixture with compound **3** (ca. 1:3 molar ratio) and its data were deduced by comparison with those reported in literature (Hirsch and Havinga, 1976). It was not observed by HPLC analysis since it is transparent at the selected wavelength (254 nm) of the

374 detector. Its presence in the irradiation mixture was confirmed by GC-MS (Figure 2) and ¹H-NMR
375 analysis of the crude irradiation mixture.

376 NMR analysis was particularly useful to examine the irradiation mixtures since loratadine and its
377 photoproducts **2-4** have characteristic identifiable signals.

378 Aqueous solutions of the drug were also exposed to sunlight in Naples (Italy) in July 2017, under
379 environmental-like conditions. As expected, degradation was slower. HPLC analysis showed a
380 decrease of drug concentration to approximately 50% after 2 days and a complex mixture of
381 photoproducts. The chromatographic and spectroscopic analysis of the irradiation mixture showed
382 the presence of photoproducts **2-4**.

383 *3.2 Mechanistic interpretation*

384 In order to gain more mechanistic information on photoproducts formation, UV-B irradiation
385 experiments were performed under various conditions (in different solvents such as acetonitrile,
386 methanol, water/acetonitrile; in the presence and absence of oxygen) and the reactions were
387 monitored by HPLC and ¹H NMR. The experimental conditions and the results are reported in
388 Table S2.

389 Accordingly with previous data (Abounassif et al., 2005), loratadine degraded faster in solutions
390 containing water (after 15 min of irradiation only 5% photodegradation in methanol or acetonitrile
391 vs. 55% in H₂O/CH₃CN 7:3 v/v, Table S2 runs **c**, **e**, **g**). In all irradiation conditions, especially
392 under argon, photoproduct **2** was present while the formation of compounds **3** and **4** was
393 observed only in aqueous solution. Control experiments showed that isoloratadine **2** was
394 photolabile and converted to unidentified material after 20 min of UV-B irradiation.

395 On the basis of literature data, a plausible mechanistic interpretation is reported in Figure 3.
396 Compound **2** should derive from a 1,3-hydrogen shift, probably via a radical pair, from an
397 excited triplet state of loratadine **1**, as suggested by the quenching with oxygen. The radical
398 recombination can give loratadine **1** or its isomer **2** (Turro et al., 2010a). Addition of water to
399 give intermediate **6** and β-cleavage of the alkoxy radical intermediate **7** should give products
400 **3** and **4**. β-Cleavage of alkoxy radicals to give ketones and stable radicals is well known (Turro et
401 al., 2010b).

402 In all the experimental irradiation conditions, desloratadine **5** was not observed. This result is
403 not surprising considering that the carbamate function is quite photostable and it does not absorb
404 light in the UV-C and UV-B regions (Iesce et al., 2006). However, control experiments showed
405 that when a 1 x 10⁻³ M solution of desloratadine **5** in H₂O/CH₃CN 7:3 v/v was irradiated by
406 UV-B lamps as reported above for loratadine, it was photodegraded within 60 min and gave a
407 complex mixture of products among which the sole identifiable product was tricycle **3**.

408 3.3 Ecotoxicological experiments

409 Tests were performed with loratadine **1**, and its photoisomer **2** and the mixture of compounds **3** and
410 **4** (in ca. 3: 1 molar ratio) obtained by preparative experiments (see 2.4.4). We also examined
411 desloratadine **5** and its photodegradation mixture (DPM) obtained as reported in 2.4.4.5.

412 3.4 Acute toxicity results

413 In order to verify that the acute effects were not DMSO-dependent, a solvent control was performed
414 for each kind of assay, at the highest tested percentage (1% v/v), and referred to the highest tested
415 concentration of 100 mg/L, observing no significant difference with the negative control, with a
416 survival higher than the 90% both in negative and in solvent controls (Table S3) as recommended
417 by test validity criteria. The parent compound loratadine, its metabolite desloratadine, the
418 transformation products and the degradation mixture of desloratadine were found to cause mortality
419 in both crustaceans and rotifers and the LC50 values, obtained after 24 h exposure (coming from
420 three independent experiments pooled using Prism5) are reported in Table 1. In addition, LC50
421 values expressed as mean \pm SD of the independent experiments are reported in Table S4. Loratadine
422 was able to cause the 50% of mortality in *C. dubia* at hundreds of μ g/L, differently from the effects
423 found at dozens of mg/L in the rotifers and in the anostracan crustacean. On the other hand, all the
424 aquatic organisms showed the same sensitivity to the metabolite, with LC50 values found at units of
425 mg/L, while DPM was more active in the rotifer *B. calyciflorus* (LC50 equal to 2.02 mg/L) than in
426 crustaceans. Transformation products such as isoloratadine **2** and the mixture of tricycle **3** and
427 piperidinone **4** showed different acute effects. In fact, albeit isoloratadine was more effective in *C.*
428 *dubia* (LC50= 1.19 mg/L), the mixture of tricycle **3** and piperidinone **4** caused 50% mortality at
429 units of mg/L not only in the cladoceran crustacean but also in the rotifer and it is more lethal than
430 parent loratadine **1** for *B. calyciflorus* (Table 1). To the best of our knowledge, scientific data on the
431 aquatic toxicity of the compounds here tested is rather scarce. **Nevertheless, there are several data**
432 **on diphenhydramine (DPH), the same histamine H1-receptor antagonist as loratadine.** In 2013
433 Goolsby and collaborators found that the diphenhydramine (DPH) was acutely toxic in *C. dubia*
434 with an LC50 value equal to 3.94 mg/L, while in 2015 Kristofco et al., found that DPH caused a
435 50% immobilisation in the cladoceran crustacean *Daphnia magna* at 374 μ g/L after 48h-exposure
436 and the 50% mortality in the fish *Danio rerio* at 45.5 mg/L after 72h exposure. In the 2011,
437 Berninger et al. found a median acute effect in *D. magna* at 0.37 mg/L after DPH exposure and
438 from units to dozens of mg/L in the fish *Pimephales promelas*. Differently from vertebrates which
439 are known to possess some degree of genetic homology for DPH targets like histamine-H1
440 receptors, with a similarity from 40 to 70% (Gunnarsson et al., 2008, Berninger et al., 2011), the
441 effects of the antihistamines in invertebrates may likely be related to other mechanisms of actions

affecting histamine ion channel transporters, as suggested by Haas et al., 2008 and Berninger et al., 2011. Regarding transformation products, they were found to be slightly toxic for all aquatic organisms tested excepted isoloratadine 2 in *C. dubia*. **Although acute toxicity data are generally very far from those of environmental concern and from the water solubility of chemicals, they are still relevant regarding the assessment of environmental risk since chronic data are often lacking.**

3.5 Chronic toxicity results

The chronic toxicity data for the five samples, reported as EC50, EC20, and EC10 values (coming from three independent experiments pooled using Prism5) and expressed in $\mu\text{g/L}$, are shown in Table 2. In addition, EC50 values expressed as mean \pm SD of the independent experiments are reported in Table S5. In order to verify that the chronic effects were not DMSO-dependent, a solvent control was performed only for *P. subcapitata* growth inhibition assay, at the highest tested percentage (0.1% v/v), and referred to the highest tested concentration of 10000 $\mu\text{g/L}$, as explained above. No significant difference with the negative control was found, with a growth higher than the 90% both in negative and in solvent controls (Table S3) as recommended by test validity criteria. Loratadine **1** was the most chronically active compound in the rotifer (EC50= 51.32 mg/L), while its metabolite desloratadine **5** was the most active both in *C. dubia*, with a median effective concentration at few units of $\mu\text{g/L}$, and in the green alga *P. subcapitata* (EC50 = 220.20 $\mu\text{g/L}$), as also depicted in Figure 4, where the concentration/effect curves of samples are reported for each aquatic organism. As shown in Figure 4, the parent compound was less active in *C. dubia* than its metabolite but more active than the transformation products. Furthermore, DPM determined a median chronic toxic effect at hundreds or thousands $\mu\text{g/L}$, in the case of aquatic consumers and producers, respectively (Table 2). In fact, as also reported in Figure 4, differently from consumers (*C. dubia* and *B. calyciflorus*), in producers (*P. subcapitata*) there is a slow increase in concentration/effect relationship with an evident response only at the highest concentrations.

In 2015 Watanabe and collaborators tested the diphenhydramine histamine H1-receptor antagonist in the alga *P. subcapitata* finding an EC50 value equal to 1240 $\mu\text{g/L}$, the same order of magnitude of the EC50 obtained in this study.

In the 2009, Isidori and collaborators tested the ranitidine, a histamine H2-receptor antagonist in the consumers *C. dubia* and *B. calyciflorus* finding a median offspring reduction in the order of thousands of $\mu\text{g/L}$, therefore underlining a higher sensitivity of these aquatic organisms to histamine H1- than to histamine H2-receptor antagonists. The environmental chronic toxicity of the histamine H1-receptor antagonist such as loratadine and its derivatives towards the aquatic tested organisms is observable already at few units-dozens $\mu\text{g/L}$ (EC20 values, Table 2), however these

residues occur in surface waters at ng/L levels which are too low to cause an immediate threat to exposed organisms but can pose delayed long term effects interfering with organism metabolic pathways.

The EC10 and EC20 values of loratadine and EC10, EC20 and EC50 values of desloratadine (Table 2) are lower than their respective water solubility equal to 11 µg/L and 3950 µg/L, making the results of this study interesting to understand the behaviour of these drugs in real water samples. At the best of our knowledge, no data of photoproducts water solubility is available.

4. Conclusion

Loratadine **1** is transformed either under UVB or by sunlight exposure. The reactive site is the double bond while the carbamate moiety is unreactive. Transformation products derive by photoisomerization and water photoaddition followed by a cleavage reaction. Photolability is also observed in desloratadine **5** but this drug leads to a complex photodegradation mixture.

The toxic effects of loratadine **1** and desloratadine **5** occur in both acute and chronic assays at concentrations higher than their environmental occurring concentrations differently affecting the organisms selected from two trophic levels. However, the environmental transformations of the parent compounds, here simulated by the UV-irradiation treatments, lead to the formation of a bioactive mixture of residues and transformation products, which could represent a harmful combination to some of the organisms tested. **In order to define water quality criteria protective for all aquatic species, further toxicity studies towards other aquatic species are needed especially to increase species sensitivity diagrams used in EU and North American approach to anti-histamine management to derive water quality benchmark, and to broaden knowledges** of mechanisms involved in the different biological responses of the organisms.

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References

- Abounassif, M.A., El-Obeid, H.A., Gadkariem, E.A., 2005. Stability studies on some benzocycloheptane antihistaminic agents. J. Pharm. Biomed. Anal. 36, 1011-1018.
- ASTM E 1440-91, 2004. Standard Guide for Acute Toxicity with the Rotifer Brachionus. American Society for Testing and Materials, Philadelphia PA, USA reapproved.

511 Berninger, J.P., Brooks, B.W., 2010. Leveraging mammalian pharmaceutical toxicology and
 512 pharmacology data to predict chronic fish responses to pharmaceuticals. *Toxicol. Lett.* 193, 69-
 513 78.
 514 Berninger, J.P., Du, B., Connors, A.K., Eytcheson, S.A., Kolkmeier, M.A., Prosser, K.N., Valenti Jr,
 515 T.V., Chambliss, K.C., Brooks, B.W., 2011. Effects of the antihistamine diphenhydramine
 516 on selected aquatic organisms. *Environ. Toxicol. Chem.* 30, 2065-2072.
 517 DellaGreca, M., Isidori, M., Temussi, F., 2014. Toxicity and Risk of Transformation Products of
 518 Emerging Contaminants for Aquatic Organisms: Pharmaceutical Case Studies, in:
 519 Lambropoulou, D. A., Nollet, L. M. (Eds.), *Transformation Products of Emerging*
 520 *Contaminants in the Environment: Analysis, Processes, Occurrence, Effects and Risks*, Wiley,
 521 Chichester (UK), pp. 827-858.
 522 Dulin, D., Mill, T., 1982. Development and evaluation of sunlight actinometers. *Environ. Sci.*
 523 *Technol.* 16, 815-820.
 524 **El-Awady M., Bela, F., Pyell, U. 2013. Robust analysis of the hydrophobic basic analytes**
 525 **loratadine and desloratadine in pharmaceutical preparations and biological fluids by**
 526 **sweeping—cyclodextrin-modified micellar electrokinetic chromatography. J.**
 527 **Chromatogr. A 1309, 64-75.**
 528 **Elyashberg, M. 2015. Identification and structure elucidation by NMR spectroscopy. Trends**
 529 **Analyt. Chem. 69, 88-97.**
 530 **Fuloria, N. K., Fuloria, S. 2013. Structural Elucidation of Small Organic Molecules by 1D, 2D**
 531 **and Multi Dimensional-Solution NMR Spectroscopy. J. Anal. Bioanal. Tech. S11: 001.**
 532 **DOI: 10.4172/2155-9872.S11-001.**
 533 Goolsby, E.W., Mason, C.M., Wojcik, J.T., Jordan, A.M., Black, M.C., 2013. Acute and chronic
 534 effects of diphenhydramine and sertraline mixtures in *Ceriodaphnia dubia*. *Environ. Toxicol.*
 535 *Chem.* 32, 2866-2869.
 536 Gunnarsson, L., Jauhiainen, A., Kristiansson, E., Nerman, O., Joakim Larsson, D.G., 2008.
 537 Evolutionary conservation of human drug targets in organisms used for Environmental risk
 538 assessments. *Environ. Sci. Technol.* 42, 5807-5813.
 539 Haas, H.L., Sergeeva, O.A., Selbach, O., 2008. Histamine in the Nervous System. *Physiol. Rev.* 88,
 540 1183-1241.
 541 **Hesse, M., Meier, H., Zeeh, B. 2008. Spectroscopic Methods in Organic Chemistry. Thieme,**
 542 **Stuttgart (DE).**
 543 Hirsch, J.A., Havinga, E., 1976. The 1-hetero-4-cyclohexanone system. Proton and carbon-13
 544 magnetic resonance, transannular effects, and conformational analysis. *J. Org. Chem.* 41, 455-
 545 462.
 546 Iesce, M.R., DellaGreca, M., Cermola, F., Rubino, M., Isidori, M., Pascarella, L., 2006. Transformation
 547 and ecotoxicity of carbamic pesticides in water. *Environ. Sci. Pollut. Res. Int.* 13, 105-109.
 548 Isidori, M., Lavorgna, M., Russo, C., Kundi, M., Žegura, B., Novak, M., Filipiè, M., Mišič, M.,
 549 Knasmueller, S., de Alda, M.L., Barceli, D., Žonja, B., Eesen, M., Šeàanèar, J., Kosjek, T.,
 550 Heath, E., 2016. Chemical and toxicological characterisation of anticancer drugs in hospital and
 551 municipal wastewaters from Slovenia and Spain. *Environ. Pollut.* 219, 275-287.
 552 Isidori, M., Parrella, A., Pistillo, P., Temussi, F., 2009. Effects of ranitidine and its photoderivatives
 553 in the aquatic environment. *Environ. Int.* 35, 821-825.
 554 ISO 14380, 2011. Water Quality-Determination of the Acute Toxicity to *Thamnocephalus Platyurus*
 555 (Crustacea, Anostraca). International Organization for Standardization, Geneva, Switzerland.
 556 ISO 20665, 2008. Water Quality-determination of Chronic Toxicity to *Ceriodaphnia Dubia* in 7
 557 Days-population Growth Inhibition Test. International Organization for Standardization,
 558 Geneva, Switzerland.
 559 ISO 20666, 2008. Water Quality-determination of Chronic Toxicity to *Brachionus Calyciflorus* in
 560 48 H-population Growth Inhibition Test. International Organization for Standardization,
 561 Geneva, Switzerland.

562 Kosonen, J., Kronberg, L., 2009. The occurrence of antihistamines in sewage waters and in
 563 recipient rivers. *Environ. Sci. Pollut. Res. Int.* 16, 555-564.

564 Kristofco, L.A., Brooks, B.W., 2017. Global scanning of antihistamines in the environment: analysis
 565 of occurrence and hazards in aquatic systems. *Sci. Total Environ.* 592, 477-487.

566 Kristofco, L.A., Du, B., Chambliss, C.K., Berninger, J.P., Brooks, B.W., 2015. Comparative
 567 Pharmacology and Toxicology of Pharmaceuticals in the Environment: Diphenhydramine
 568 Protection of Diazinon Toxicity in *Danio rerio* but not *Daphnia magna*. *The AAPS Journal* 17,
 569 175-183.

570 **Lambropoulou, D. A., Nollet, L. M. 2014. Transformation Products of Emerging**
 571 **Contaminants in the Environment: Analysis, Processes, Occurrence, Effects and Risks,**
 572 **Vol. 1 and 2, Wiley, Chichester (UK),**

573 **Li, M.-H., 2012. Survival, mobility, and membrane-bound enzyme activities of**
 574 **freshwater planarian, *dugesia japonica*, exposed to synthetic and natural surfactants.**
 575 ***Environ. Toxicol. Chem.* 31 (4), 843-850.**

576 López-Serna, R., Petrovic, M., Barceló, D., 2012. Direct analysis of pharmaceuticals, their
 577 metabolites and transformation products in environmental waters using on-line TurboFlow™
 578 chromatography–liquid chromatography–tandem mass spectrometry. *J. Chromatogr. A.* 1252,
 579 115-129.

580 **Moreno-González, R., Rodriguez-Mozaz, S., Gros, M., Barceló, D., León, D.M. 2015. Seasonal**
 581 **distribution of pharmaceuticals in marine water and sediment from a mediterranean**
 582 **coastal lagoon (SE Spain). *Environ. Res.* 138, 326-344.**

583 OECD 201, 2011. Guideline for the Testing of Chemicals 201: Freshwater Alga and Cyanobacteria,
 584 Growth Inhibition Test. Organization for Economic Cooperation and Development.

585 Paixao, S.M., Silva, L., Fernandes, A., O'Rourke, K., Mendonca, E., Picado, A., 2008. Performance
 586 of a miniaturized algal bioassay in phytotoxicity screening. *Ecotoxicology* 17, 165-171.

587 Parfitt, K., 1999. Martindale: The extra Pharmacopoeia (32nd ed). Pharmaceutical Press: London, 413-
 588 449.

589 Passananti, M., Lavorgna, M., Iesce, M. R., DellaGreca, M., Brigante, M., Criscuolo, E., Cermola,
 590 F., Isidori, M., 2015. Photochemical fate and eco-genotoxicity assessment of the drug etodolac.
 591 *Sci. Total Environ.* 518-519, 258-265.

592 Peyrovi, M., Hadjmohammadi, M., 2015. Extraction optimization of Loratadine by supramolecular
 593 solvent-based microextraction and its determination using HPLC. *J. Chromatogr. B* 980, 41-47.

594 **Radjenovic', J, Petrovic, M., Barcelo', D. 2009. Fate and distribution of pharmaceuticals in**
 595 **wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced**
 596 **membrane bioreactor (MBR) treatment. *Water Res.* 43, 831-841.**

597 Ramanathan, R., Reyderman, L., Kulmatycki, K., Su, A.D., Alvarez, N., Chowdhury, S.K.,
 598 Alton, K.B., Wirth, M.A., Clement, R.P., Statkevich, P., Patrick, J.E., 2007. Disposition of
 599 loratadine in healthy volunteers. *Xenobiotica* 37, 753-769.

600 Turro, N.J., Ramamurthy, V., Scaiano, J.C., 2010a. Modern Molecular Photochemistry of Organic
 601 Molecules. University Science Books, Sausalito (USA), pp. 705-728.

602 Turro, N.J., Ramamurthy, V., Scaiano, J.C. 2010b. Modern Molecular Photochemistry of Organic
 603 Molecules. University Science Books, Sausalito (USA), pp. 665-668.

604 US EPA, 1993. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to
 605 Freshwater and Marine Organisms. EPA-600-4-90, fourth ed. US Environmental Protection
 606 Agency, Washington DC.

607 **Valcarcel, Y., Gonzalez Alonso, S., Rodriguez-Gil, J. L., Gil, A., Català, M. 2011. Detection of**
 608 **pharmaceutically active compounds in the rivers and tap water of the Madrid Region**
 609 **(Spain) and potential ecotoxicological risk. *Chemosphere*, 84, 1336-1348.**

610 Valenti, T.W.J., Perez-Hurtado, P., Chambliss, C.K., Brooks, B.W., 2009. Aquatic toxicity of
 611 sertraline to *Pimephales promelas* at environmentally relevant surface water pH. *Environ.*
 612 *Toxicol. Chem.* 28, 2685-2694.

613 Watanabe, H., Tamura, I., Abe, R., Takanobu, H., Nakamura, A., Suzuki, T., Hirose, A., Nishimura,
614 T., Tatarazako, N., 2016. Chronic Toxicity Of An Environmentally Relevant Mixture Of
615 Pharmaceuticals To Three Aquatic Organisms (Alga, Daphnid, And Fish). *Environ. Toxicol.*
616 *Chem.* 35, 996-1006.

617 Witiak, D.T., 1970. Antiallergenic agents, in Burger, A. (Ed.) *Medicinal chemistry* (3rd ed). Wiley
618 Interscience, New York, Chapter 65.

619 Yumibe, N., Huie, K., Chen, K.J., Snow, M., Clement, R.P., Cayen, M.N., 1996. Identification of
620 human liver cytochrome P450 enzymes that metabolize the nonsedating antihistamine
621 loratadine. Formation of descarboethoxyloratadine by CYP3A4 and CYP2D6. *Biochem.*
622 *Pharmacol.* 51, 165-172.

623